

WHAT IS CLAIMED IS:

1. A method of identifying an animal which has been vaccinated with an immunogen comprising:
 - 5 a. combining said immunogen with a recombinant, substantially non-toxic *E. coli* heat-labile enterotoxin mutant (rmLT) to form a vaccine composition;
 - b. administering said vaccine composition to animal subjects; and
 - c. detecting the presence of antibodies or immune cells that are specific to said rmLT in said animal thereby identifying said animal as having been vaccinated
10 with said vaccine preparation.
2. A method of determining that an animal that has been vaccinated with a vaccine composition which includes an immunogen and a substantially nontoxic rmLT, said method comprising detecting, in said animal, the presence of antibodies or immune cells
15 that are specific to said rmLT.
3. A method of marking or identifying a vaccine composition containing an immunogen, comprising:
 - a. adding a substantially nontoxic rmLT in said vaccine composition;
 - 20 b. administering said vaccine composition to an animal; and
 - c. detecting presence of antibodies or T cells that are specific to said rmLT in said animal thereby identifying said vaccine composition.
4. The method according to any one of claims 1-3, wherein said animal is a food animal
25 selected from the group consisting of cattle, sheep, pig and poultry.
5. The method according to any one of claims 1-3, wherein said immunogen is a *Mycoplasma hyopneumoniae* immunogen.
- 30 6. The method according to claim 5, wherein said *Mycoplasma hyopneumoniae* immunogen comprises an inactivated, whole or partial *Mycoplasma hyopneumoniae* cell preparation.
7. The method according to claim 6, wherein said *Mycoplasma hyopneumoniae* cell preparation is RESPISURE®, RESPISURE ONE™, STELLAMUNE™ Mycoplasma or
35 STELLAMUNE ONE™ Mycoplasma.

8. The method according to any one of claims 1-3, wherein said rmLT comprises the substitution of Gly for Arg at the amino acid position 192.
- 5 9. The method according to claim 8, wherein said rmLT further comprises the substitution of Ala for Leu at the amino acid position 211.
- 10 10. The method according to any one of claims 1-3, wherein said rmLT in said vaccine composition is in an amount of about 1-500 µg per dose.
- 11 11. The method according to claim 10, wherein said rmLT in said vaccine composition is in an amount of about 20-200 µg per dose.
- 15 12. The method according to claim 11, wherein said rmLT in said vaccine composition is in an amount of about 100 µg per dose.
- 13 13. The method according to claim 1 or 3, wherein the administration of said vaccine composition is via an oral, intranasal, mucosal, topical, transdermal, or parenteral route.
- 20 14. The method according to claim 13, wherein the administration of said vaccine composition is via an intramuscular route.
- 15 15. The method according to any one of claims 1-3, wherein said antibodies specific to said rmLT are detected in blood or milk of said animal or in meat juice from said animal after processing.
- 25 16. The method according to claim 15, wherein said antibodies are detected in an immunoassay.
- 30 17. The method according to claim 16, wherein said immunoassay is in the form of DIPSTICK, Western Immuno Blot or ELISA.
- 35 18. The method according to claim 16, wherein a peptide fragment of the A subunit of said rmLT is used in said assay.

19. The method according to claim 18, wherein said peptide fragment is selected from any one of SEQ ID NOS: 1-9.
20. A method for enhancing the immunoprotective effects of an immunogen in a vaccine composition for administration to an animal, comprising adding to said vaccine composition a substantially nontoxic rmLT.
21. The method according to claim 20, wherein an oil-in-water emulsion adjuvant is further added to the vaccine composition.
22. The method according to claim 21, wherein said oil-in-water emulsion adjuvant is AMPHIGEN®.
23. The method according to claim 20, wherein said animal is a food animal selected from the group consisting of cattle, sheep, pig, and poultry.
24. The method according to claim 20, wherein said immunogen is a *Mycoplasma hyopneumoniae* immunogen.
25. The method according to claim 24, wherein said *Mycoplasma hyopneumoniae* immunogen comprises an inactivated, whole or partial *Mycoplasma hyopneumoniae* cell preparation.
26. The method according to claim 25, wherein said *Mycoplasma hyopneumoniae* cell preparation is RESPISURE®, RESPISURE ONE™, STELLAMUNE™ Mycoplasma or STELLAMUNE ONE™ Mycoplasma.
27. The method according to claim 20, wherein said rmLT comprises the substitution of Gly for Arg at the amino acid position 192.
28. The method according to claim 27, wherein said rmLT further comprises the substitution of Ala for Leu at the amino acid position 211.
29. The method according to claim 20, wherein said rmLT in said vaccine composition is in an amount of about 1-500 µg per dose.

30. The method according to claim 29, wherein said rmLT in said vaccine composition is in an amount of about 20-200 µg per dose.

5 31. The method according to claim 30, wherein said rmLT in said vaccine composition is in an amount of about 100 µg per dose.

32. The method according to claim 20, wherein the administration of said vaccine composition is via an oral, intranasal, mucosal topical, transdermal, or parenteral route.

10 33. The method according to claim 32, wherein the administration of said vaccine composition is via an intramuscular route.

34. A vaccine composition for administration to an animal comprising an immunogen, a substantially non-toxic rmLT and an oil-in-water emulsion adjuvant.

15 35. The vaccine composition of claim 34, wherein said oil-in-water emulsion adjuvant is AMPHIGEN®.

20 36. The vaccine composition according to claim 34, wherein said animal is a food animal selected from the group consisting of cattle, sheep, pig, and poultry.

37. The vaccine composition according to claim 34, wherein said immunogen is a *Mycoplasma hyopneumoniae* immunogen.

25 38. The vaccine composition according to claim 37, wherein said *Mycoplasma hyopneumoniae* immunogen comprises an inactivated, whole or partial *Mycoplasma hyopneumoniae* cell preparation.

30 39. The vaccine composition according to claim 38, wherein said *Mycoplasma hyopneumoniae* cell preparation is RESPISURE®, RESPISURE ONE™, STELLAMUNE™ *Mycoplasma* or STELLAMUNE ONE™ *Mycoplasma*.

35 40. The vaccine composition according to claim 34, wherein said rmLT comprises the substitution of Gly for Arg at the amino acid position 192.

41. The vaccine composition according to claim 40, wherein said rmLT further comprises the substitution of Ala for Leu at the amino acid position 211.